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EFFECTS OF PLANT FLAVONOIDS ON FECUNDITY, SURVIVAL, AND FEEDING OF THE FORMOSAN SUBTERRANEAN TERMITE

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Abstract—Fecundity, mortality, and food consumption of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, were evaluated in response to five plant flavonoids (genistein, biochanin A, apigenin, quercetin, and glyceollin). Apigenin fed at 50 μg/primary reproductive pair proved to be the most toxic flavonoid. Biochanin A was most effective in reducing fecundity. Subsequently, these two flavonoids were tested through oral feeding and topical application at 100-μg dose. Significant reduction in the numbers of progeny was evident for biochanin A in both treatment methods. Choice feeding tests with termite workers showed that initially termites were attracted to filter paper treated with biochanin A, but over a period of 72 hr, consumed significantly less material when compared to controls. Biochanin A is a promising phytochemical with ability to reduce fecundity in primary reproductives of the Formosan subterranean termite, but it does not elicit phagostimulant activity.

Key Words—*Coptotermes formosanus*, feeding, fecundity, termites, flavonoid, flavone, isoflavone, genistein, biochanin A, apigenin, quercetin, glyceollin, survival.

INTRODUCTION

Flavonoids are phenolic compounds commonly found in many plants, vegetables, and flowers. Although flavonoids are ubiquitous, isoflavones are found only in a few legumes, particularly soybean. Flavonoids are able to modulate growth and reproduction of herbivores by directly interacting with steroid hormone systems

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(Oberdorster et al., 2001). In insects, several classes of phytochemicals, including the flavonoids, interfere with molting, reproduction, feeding, and behavior (Reyes-Chilpa et al., 1995; Musayimana et al., 2001; Simmonds, 2001). Insecticidal activity of flavonoids has been shown against the western corn rootworm (Mullin et al., 1992), the corn earworm (Widstrom and Snook, 2001), and the common cutworm (Morimoto et al., 2000). Reyes-Chilpa et al. (1995) determined that two flavonoids, castillen D and castillen E, showed concentration-dependent feeding deterrence, but were not toxic to *Cryptotermes brevis*. In a review of chemicals found to impart natural resistance in wood, Rao (1982) reported that flavonoids, including isoflavonoids and neoflavonoids, were important.

Coptotermes formosanus Shiraki is a serious urban pest causing extensive damage to wooden structures and live trees in several of the southern states and Hawaii in the United States. To date, no studies have examined sustainable management of *C. formosanus*, using inexpensive phytochemicals, including flavonoids. Flavonoids have been shown to interact with the ecdysone receptor using a reporter-gene assay and ecdysone-responsive cell lines (Oberdorster et al., 2001). Considering the ability of flavonoids to bind with the ecdysone receptor, flavonoids may affect other biological systems in termites. In this study, effects of oral administration of five selected flavonoids on mortality and fecundity of primary reproductives of the Formosan subterranean termite were determined. The five flavonoids (Figure 1) tested include the isoflavones glyceollin, genistein, and biochanin A, and the flavones apigenin and quercetin. In a subsequent test, only biochanin A and apigenin were evaluated after oral and topical application. The two flavonoids were also tested for feeding preference by termite workers in a no-choice and choice tests.

METHODS AND MATERIALS

Chemicals. Apigenin, genistein, quercetin, and biochanin A were obtained from Indofine Chemical (Somerville, NJ). Glyceollin (mixture of glyceollins I, II, III in a 6:2:1 ratio) was isolated using a procedure developed in this center (Burow et al., 2001). Soybean seeds (*G. max* cv. Pioneer 95B41, 50 g) were sliced and inoculated with *Aspergillus sojae*. After 3 days, glyceollin was extracted from the inoculated seeds with 80% ethanol. Glyceollin was isolated using preparative scale HPLC and confirmed by UV–vis spectrophotometry and electrospray mass spectrometry. Solvents [acetone and dimethyl sulfoxide (DMSO)] were purchased from Sigma-Aldrich (Milwaukee, WI). Water treated with a Millipore system was used during sample preparation procedures and HPLC analyses.

Insects. In Louisiana, *C. formosanus* swarm during April–June each year. Alates or primary reproductives were collected in 2001 and 2002 around light traps set up in New Orleans, LA. The dealates after losing their wings were sexed and used in the tests within a day. Termite workers were collected from ground

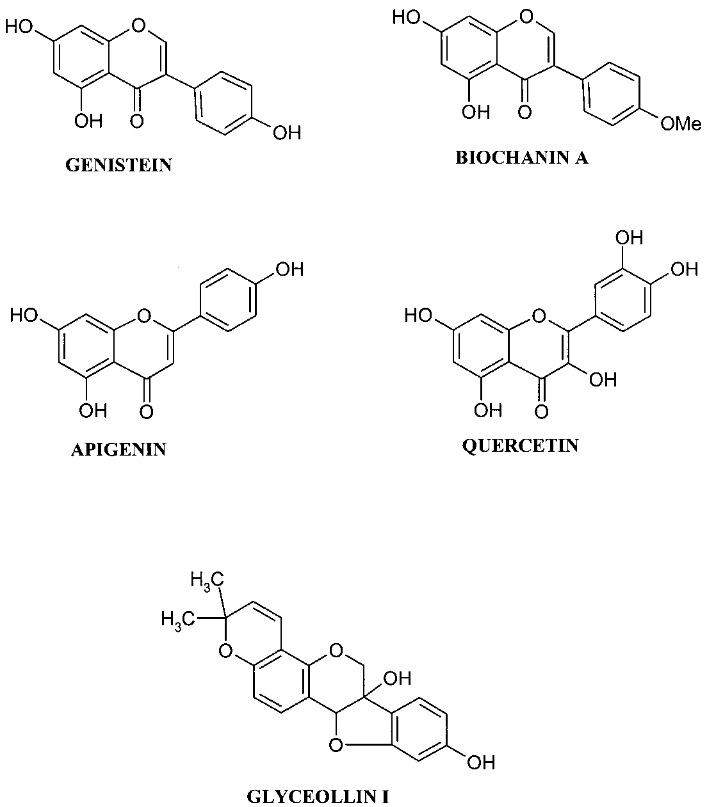

FIG. 1. Flavonoid/isoflavonoid structures. Only the structure of the predominant glyceollin isomer, glyceollin I, is shown.

traps setup in the City Park area, brought to the laboratory, and fed on pieces of pine wood in an incubator maintained at 28°C and 70% RH, until they were used in the tests.

Fecundity Bioassays. An initial test with five flavonoids was carried out in 2001. Except for apigenin, the chemicals (50 μg), were dissolved in acetone (5 μl) and applied to 12.2-mg bait matrix (0.41% by weight; bait matrix composition described in Rojas and Morales-Ramos, 2001). Apigenin was predissolved in DMSO before making the acetone solution (10% DMSO). Twenty pairs of dealates together with the treated bait matrix were placed singly in tight fitting, 50 × 9 mm Falcon Petri dishes (Becton Dickinson, NJ) containing agar–sawdust medium (Raina et al., 2003). Control insects were provided with bait matrix treated with 5-μl acetone. Observations on mortality and fecundity were recorded 30 days after treatment.

On the basis of the results from the first test, a second test was conducted in 2002. Only biochanin A and apigenin were used in both oral and topical applications. For oral application, Biochanin A (100 μg) was dissolved in acetone (5-μl) and applied to a 10-mg piece of Kimwipes™ (Kimberly-Clark, GA). Apigenin was predissolved in DMSO before making the acetone solution (10% DMSO). After allowing the solvent to evaporate, the Kimwipe™was treated with distilled water (10-μl) before being placed into a 50×9 mm Petri dish together with a pair of dealates. Thirty dealate pairs were allowed to feed for 72 hr, then transferred to Petri dishes containing diet as described above. Observations on mortality and total progeny were recorded after 30 days. Control insects were fed on Kimwipes™ treated with acetone followed by water. For topical applications of biochanin A and apigenin, each chemical was dissolved in DMSO at a concentration of 10 μg/0.1 μl. The solution (0.1 μl) was applied to both males and females after cooling them on ice. Thirty dealate pairs were tested and observations were recorded after 30 days.

Attractant and Feeding Bioassays. Only biochanin A was tested with termite workers in no-choice and choice tests. The tests were conducted in plastic Petri dishes (90×18 mm), each filled with sand (15-g) moistened with distilled water (1.5-ml). Filter papers (Whatman Grade 5, 2.5 cm diam) were weighed and treated with 50 μl of a 1% (w/w) acetone solution of biochanin A and then air-dried. Control filter papers were treated with 50-μl acetone. For the no-choice test, the treated and control filter papers were placed in separate Petri dishes with 100 workers per dish. Test dishes were placed in an incubator for 72 hr. In the choice test, a treated and a control filter paper disk were both placed in the same Petri dish with 200 workers per dish. Distribution of the termites was visually monitored for 1 hr, photographed after 1 hr, and the Petri dishes were placed into the incubator for 72 h. After this period, the filter papers were removed, washed, oven-dried (60°C for 1 hr), and weighed to determine consumption. The test was replicated three times.

Data Analysis. Significance tests, comparing treatments with controls for each experiment, were evaluated by a one-way Analysis of Variance (ANOVA) using a Dunnett's Multiple comparison posttest at the 5% level. Statistical tests were run on Prism software (GraphPad Prism version 2.1, San Diego, CA.)

RESULTS

Fecundity and Mortality. Addition of flavonoids did not appear to deter the primary reproductives from feeding on the bait matrix, with low to moderate feeding in all the treatments including control. The fecundity results from an initial screening experiment using the five flavonoids at 50 μg added to the bait matrix are presented in Figure 2. Only biochanin A and genistein significantly decreased the number of progeny (29.5 and 18.6%, respectively) produced from each alate pair after 30 days when compared to untreated controls. There was no effect of any of the flavonoids on egg hatch (data not shown). Apigenin was

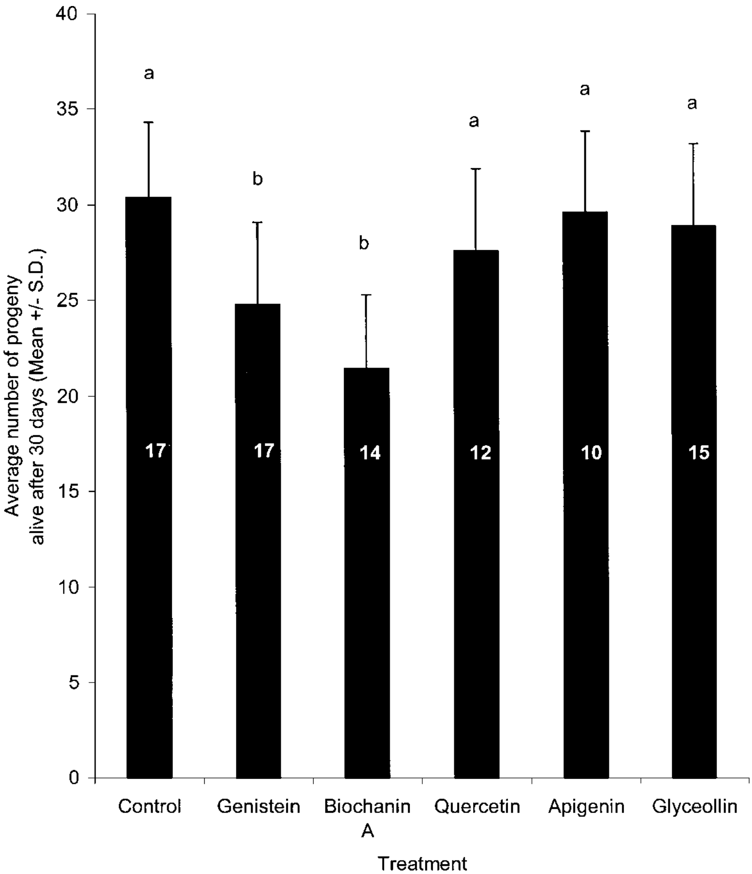

FIG. 2. Fecundity of primary reproductives of the Formosan subterranean termite 30 days following feeding on five flavonoids (50 μg). Bars with different letters are significantly different ($P < 0.01$, Dunnett's multiple comparison test). Numbers within bars indicate the sample size.

the most toxic flavonoid tested, resulting in 50% mortality among the primary reproductives (Table 1). Quercetin caused 40% mortality compared to 20% in the control group.

Biochanin A and apigenin were selected for further testing using 100-μg and 10-μg doses for oral and topical applications, respectively. In the oral treatment, total progeny after 30 days was significantly lower in both biochanin-A- and apigenin-treated groups compared to control (Figure 3). Of the two treatments, biochanin A was superior, causing about 32% decrease in the progeny. With topical application, only biochanin A caused a significant decrease in the progeny, and

TABLE 1. EFFECT OF ORAL FEEDING AND TOPICAL APPLICATION OF SELECTED PLANT FLAVONOIDS ON MORTALITY AMONG PRIMARY REPRODUCTIVES OF THE FORMOSAN SUBTERRANEAN TERMITE

	Percent mortality (N)		
Treatment	Oral 50 μg[a]	Oral 100 μg[b]	Topical 10 μg
Control	20 (17)	20 (30)	26.7 (30)
Genistein	15 (17)	—	—
Biochanin A	30 (14)	26.7 (30)	36.7 (30)
Quercetin	40 (12)	—	—
Apigenin	50 (10)	33.3 (30)	30 (30)
Glyceollin	25 (15)	—	—

[a] Flavonoids except apigenin were dissolved in acetone and applied to 12.2 mg bait matrix. Apigenin was predisolved in DMSO. Observations were recorded after 30 days.
[b] Flavonoids dissolves as above and applied to 10-mg Kimwipes™ tissue.

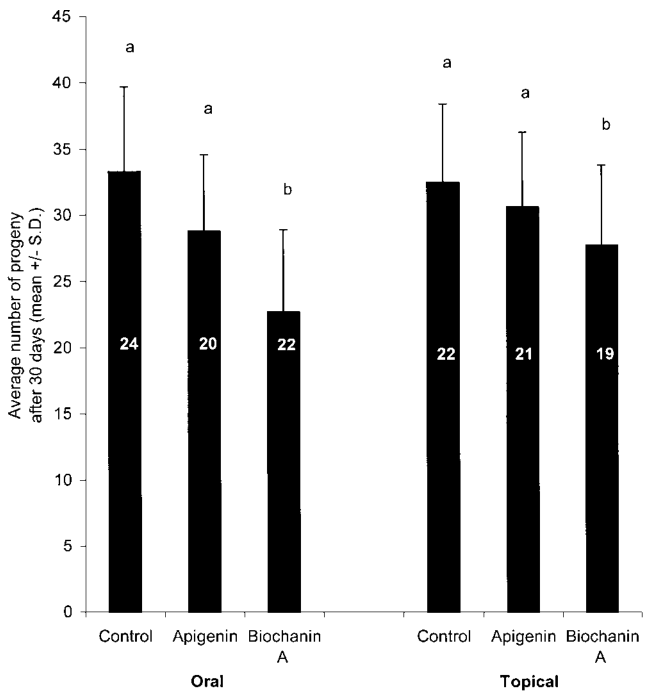

FIG. 3. Fecundity of primary reproductives of the Formosan subterranean termite 30 days following oral feeding (100 μg) or topical application (10 μg) with apigenin and biochanin A. Bars within a treatment method with different letters are significantly different ($P < 0.05$, Dunnett's Multiple comparison test). Numbers within bars indicate the sample size.

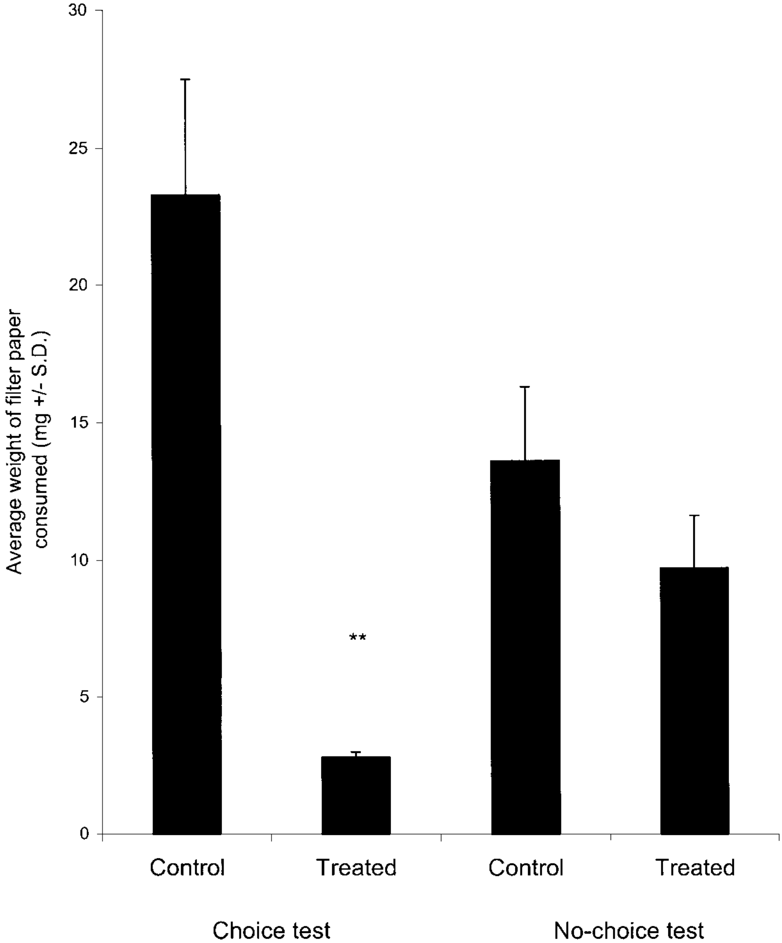

FIG. 4. Consumption of paper in no-choice and choice tests using biochanin A (50 μl). Means ($N = 3$) using 100 Formosan subterranean termite workers in no-choice test, and means ($N = 3$) using 200 workers in choice test. No significant difference versus acetone control was observed in no-choice test ($P > 0.05$), however in a choice test biochanin A was significantly different (**) versus control at $P < 0.01$ (Dunnett's Multiple comparison test).

the effect was lower (14.8% reduction) as compared to the oral method. Mortality caused by the two flavonoids ranged from 26.7 to 36.7%, which was not significantly higher than in the control groups (Table 1). The average consumption of paper in this experiment was 25% for the control group, 40% for the group treated with biochanin A, and 17.5% for the group treated with apigenin.

Preference Tests. Only biochanin A was tested in both no-choice and choice tests using *C. formosanus* workers. In the choice test, a significantly higher amount (88%) of the control paper was consumed in 72 hr, when compared to the consumption of biochanin-A-treated paper (Figure 4). Even though the workers were initially highly attracted to the flavonoid-treated paper when no choice was provided, consumption was not different between control paper as compared to biochanin-A-treated paper (Figure 4).

DISCUSSION

Flavonoids are numerous and widespread among natural plant constituents and serve many functions, including plant defense, pigmentation, and many diverse host–plant interactions. Most plants contain an array of flavonoids, and evidence has been presented suggesting that insects are able to discriminate among plants with varying flavonoid profiles (Schoonhoven, 1982; Feeny et al., 1988; Simmonds, 2001). Certain flavonoids either stimulate insect feeding (Bernays et al., 1991) or act as feeding deterrents (Morimoto et al., 2000). They can act as endocrine disruptors in mammalian systems, having high binding affinities for estrogen receptors, and have recently been shown to bind the ecdysone receptor of insects (Oberdorster et al., 2001). Although several studies have examined the effects of natural bioactive products on feeding, behavior, mortality, and fecundity of termites (Chang et al., 2001; Tellez et al., 2001; Zhu et al., 2001), only one study (Reyes-Chilpa et al., 1995) has examined the effects of flavonoids on termite behavior.

Of the flavonoids tested at 50 μg, only the isoflavones genistein and biochanin A resulted in a significant reduction in the number of progeny produced within 30 days following treatment. The flavones apigenin and quercetin, in contrast, had no significant effect on fecundity. However, the flavones caused higher mortality among the primary reproductives. In endocrine disruptor studies, isoflavones are more active when compared to flavones, particularly in their ability to bind to the estrogen receptor in mammalian systems and have been shown to disrupt animal reproduction (Shutt, 1976; Setchell et al., 1987). The isoflavone glyceollin is synthesized in soybean during pathogen attack and has antifungal properties, but is a weakly active endocrine disruptor (Burow et al., 2001). It showed little activity at inhibiting fecundity or increasing mortality among primary reproductives.

In the experiments that focused on the effects of biochanin A and apigenin on fecundity and mortality following oral feeding at 100 μg/pair and topical application at 10 μg/individual, biochanin A was again more active at decreasing egg-laying ability of the primary reproductives in both oral and topical treatments. After confirming the ability of biochanin A to lower fecundity in the primary reproductives, no-choice and choice tests were conducted to examine the flavone's attractant and phagostimulant activities. There is a paucity of research on the effects of flavonoids on insect, particularly termite, feeding and toxicity. Several flavones are phagostimulants to many polyphagous insects (Bernays et al., 1991; Harborne and Grayer, 1994). Choice feeding tests with castillen D and E, isolated from the heartwood of the tropical tree *Lochocarpus castilloi*, showed concentration-dependent feeding deterrent activity, but they were not toxic to *Cryptotermes brevis* (Reyes-Chilpa et al., 1995). Our results showed that in a choice test, although termite workers were initially attracted to the filter paper treated with biochanin A, within 1 hr they dispersed evenly between the treated and control papers. After 72 hr, termites had consumed less of the treated filter paper. Consequently, no phagostimulant activity was observed. In order for biochanin A to be incorporated into bait matrix, a phagostimulant must be added to enhance the effectiveness of the bait matrix.

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